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A Versatile Approach to Reducible Vinyl Polymers via Oxidation of Telechelic Polymers Prepared by Reversible Addition Fragmentation Chain Transfer Polymerization

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Introduction

Synthesis of biodegradable polymers has received significant attention due to its importance in a range of applications, including sophisticated biomedical devices and prevention of environmental pollution.[1] Biodegradable polymer materials usually contain chemical bonds that can be easily cleaved chemically or biologically under mild conditions.[2−7] Disulfide-containing reducible polymers represent an attractive alternative to hydrolyzable polymers in the preparation of biodegradable materials.[8−12] The disulfide groups can be cleaved in the presence of reducing agents, and the redox potential of the disulfide/thiol couple can be easily controlled by the reaction conditions. These disulfide-containing reducible polymers can be synthesized either by an oxidation of dithio-functionalized polymer precursors or by using disulfide-containing monomers or cross-linkers.[4,8,9,11−16] Interest in the reducible polymers is currently driven mainly by their favorable properties in drug and gene delivery applications and many of the practically used reducible polymers are based on peptides.[17,18]

Vinyl polymers represent one of the most important classes of polymer materials currently in use. Many of them have potential applications in molecular imaging, nanomedicine, and drug and gene delivery. However, because of the stability of their C−C backbone, these polymers are not readily susceptible to biodegradation, which significantly limits their biomedical applications. Introducing biodegradable disulfide bonds into vinyl polymers has therefore potential to become an important area of research. Recently, Matyjaszewski et. al and Armes et. al used atom transfer radical polymerization of disulfide-based divinyl monomers to prepare degradable gels and disulfide-based initiators to prepare a linear polymer containing a disulfide bond in the center.[10,19] However, synthesis of reducible vinyl polymers containing multiple disulfide bonds in the backbone has been seldom explored until now.

Reversible addition fragmentation chain transfer (RAFT) polymerization is an extremely versatile, controlled free radical polymerization technique for the synthesis of well-defined polymer architectures with narrow molecular weight polydispersity.[20−26] The RAFT process is applicable to a wide range of monomers and can be performed under a broad range of conditions. On the basis of the mechanism of RAFT polymerization, RAFT polymerization using a difunctional RAFT agent yields macromolecules containing transfer agent residues at both ends of the polymer chain, which can be further converted into terminal thiol groups.[27−29] The polymers obtained with difunctional agents will contain a very limited amount of the "dead" polymer chains as long as the RAFT agent is present in sufficient excess over the initiator used.[29]

The goal of this study was to expand the portfolio of available reducible polymers with vinyl polymers by utilizing the prior findings with RAFT polymerization using difunctional transfer agents.[27−29] As this study was being prepared for publication, the synthesis of reducible poly(St) was reported by Monteiro and colleagues.[30] Here we report the synthesis of high-molecular-weight reducible linear polymers based on three different monomers: styrene (St), acrylic acid (AA), and 2-dimethylaminoethyl methacrylate (DMAEMA).

Experimental Section

Materials. 2,2′-Azobis(2-methylpropionitrile) (AIBN, Sigma-Aldrich 98%), hexylamine (Aldrich, 99%), 1,4-diisopropenylbenzene (TCI), sulfur (EMD, 99%), anhydrous methanol (EMD, 99.8%), sodium methoxide (Alfa, 25−30%), benzyl chloride (Alfa, 99%), carbon tetrachloride (Sigma, 99%), tetrahydrofuran (THF, VWR, 99%), anhydrous diethyl ether (EMD, 99.0%), hexane (EMD, 98.5%), and dithiothreitol (Fluka, >99%) were used as received. Styrene (TCI, 99%) and acrylic acid (TCI, 99%) were distilled to remove inhibitor and stored at 4 °C before use. 2-(Dimethylamino)ethyl methacrylate (DMAEMA, Acros, 99%) was passed through a column of activated basic alumina to remove an inhibitor and stored at 4 °C before use.

Instrumentation. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian spectrometer (400 MHz). The number-average (M_n) and weight-average (M_w) molecular weight and polydispersity index (PDI, M_w/M_n) of the polymers were determined by size exclusion chromatography (SEC) using a Shimadzu LC-10ADVP liquid chromatograph equipped with a CTO-10ASVP Shimadzu column oven and a Polymer Labs PL gel 5 μm mixed C column. The system was equipped with a seven-angle BIMwA static light scattering detector and a BIDNDC differential refractometer (both from Brookhaven Instruments, Inc.). The BIMwA detector was equipped with a 30 mW vertically polarized solid-state laser (660 nm) as a light source. *N*,*N*-Dimethylformamide (DMF) was used as an eluent at a flow rate of 1.0 mL/min and a temperature of 35 °C. SEC data were analyzed using PSS WinGPC Unity software from Polymer Standards Services. Refractive index increments (d*n*/d*c*) of the polymers were determined by a BIDNDC differential refractometer (Brookhaven Instruments, Inc.) and used in the SEC analysis.

Synthesis of Dithiobenzoic Acid (DTBA).[31] Sodium methoxide (25−30% solutions in methanol, 100.0 g, ∼0.50 mol) was added to a 500 mL, three-neck round-bottomed flask. Anhydrous methanol (160.0 mL) was added to the flask, followed by rapid addition of elemental sulfur (16.0 g, 0.50 mol). Benzyl chloride (31.5 g, 0.25 mol) was then added dropwise via an addition funnel over a period of 60 min at room temperature under a dry nitrogen atmosphere. The reaction mixture was heated in an oil bath at 70 °C for 18 h. Then, the reaction mixture was cooled to 5 °C using an ice bath. The precipitated salt was removed by filtration, and then methanol was removed. Water (500 mL) was added to the residue, and the crude sodium dithiobenzoate solution was washed three times with diethyl ether. Diethyl ether and 1.0 M HCl (250 mL) were added, DTBA was extracted into the ether layer, and the ether layer was washed three times with water. Water (200 mL) and 1.0 M NaOH (250 mL) were added, and sodium dithiobenzoate was extracted into the aqueous layer and washed three times with diethyl ether. Then, diethyl ether and 1.0 M HCl (250 mL) were added, DTBA

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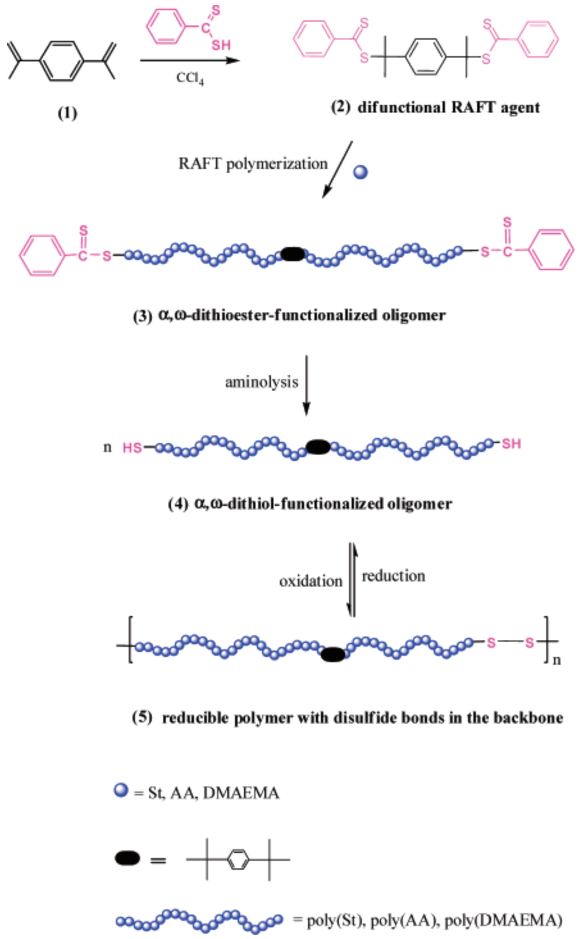
Scheme 1. Synthesis of Reducible Polymers Containing Multiple Disulfide Bonds in the Backbone via Oxidation of Telechelic Polymers Prepared by RAFT Polymerization



was extracted into the ether layer, washed the ethereal layer with water thre times, and dried by anhydrous Na_2SO_4, and the volatiles were removed under reduced pressure. Dithiobenzoic acid was obtained as an oil with 38% yield. ^{1}H NMR ($CDCl_3$, δ): 8.0 (2H, aromatic protons next to thioester), 7.6 (1H, aromatic protons), 7.4 (2H, aromatic protons), 6.3 (1H, -CSS*H*).

Synthesis of 1,4-Bis(2-(thiobenzoylthio)prop-2-yl)benzene (BTBTPB).[32] A mixture of DTBA (14.0 g, 0.091 mol) and 1,4-diisopropenylbenzene (6.5 g, 0.041 mol) in CCl_4 (100 mL) was heated at 80 °C for 24 h. The volatiles were removed under reduced pressure, and the residue was triturated with 1:4 diethyl ether/hexanes to isolate the product as a pink solid (26% yield). ^{1}H NMR ($CDCl_3$, δ): 2.0 (12H, s), 7.3 (m, 4H), 7.47 (m, 6H), 7.84 (m, 4H). ^{13}C NMR ($CDCl_3$, δ): 227, 146.5, 142.6, 132,128.3, 126.5, 126.3, 56.2, 28.2.

General Procedure for Polymerization. All polymerizations were performed in ampoules under homogeneous conditions. A typical polymerization was initiated by an addition of AIBN. (The molar ratio of the RAFT agent to AIBN was >20.) Solutions of the respective monomer, initiator, and RAFT agent in THF were added into the ampoule. The mixtures were thoroughly deoxygenated, sealed under vacuum, and placed in a thermostated water bath at 60 °C. After a predetermined reaction time, the ampoule was opened, and the polymerization was quenched by lowering the temperature to 0 °C. The resulting polymer was obtained by precipitation and filtration.

Conversion of the Dithioester End Groups into Thiol Groups. For the preparation of α,*ω*-dithio-functionalized St and DMAEMA precursors, the general procedure was as follows. The α,*ω*-bis-(dithioester)-functionalized polymers were dissolved in THF containing a few drops of aqueous sodium bisulfite. The reaction mixture was purged of oxygen by bubbling with N_2 for 30 min, hexylamine was then added, and the reaction was stirred for 3 h under N_2. The solution color changed from pink to light yellow during the aminolysis, which indicated the conversion of dithioester groups into thiol functionalities. The reaction mixture was then added dropwise to a 10-fold excess of diethyl ether, and α,*ω*-dithio-functionalized polymers were collected by filtration.

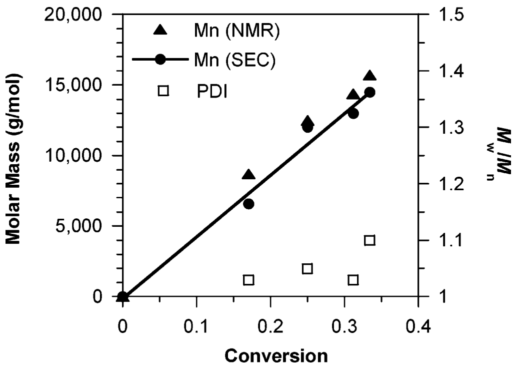


Figure 1. Effect of conversion on the changes of molar mass during RAFT polymerization of St using a difunctional RAFT agent.

Table 1. Typical Results of the Oxidation and Reduction of the Synthesized Polymers

	α,*ω*-dithio precursor				M_n	
sample	M_n	M_w/M_n	oxidation solvent	oxidation agent	after oxidation	after reduction
St	3500	1.05	THF	I_2	35 200	4100
DMAEMA	2900	1.20	water	DMSO	21 000	2600
AA	2500	1.15	THF	I_2	32 000	2900

Because of hexylamine salt formation, the α,*ω*-bis(dithioester)-functionalized poly(AA) was dissolved in 0.1 M NaOH solution, and the reaction mixture was stirred for 10 h under N_2. The solution's pink color faded gradually during the reaction, indicating the hydrolysis of α,*ω*-bis(dithioester)-functionalized poly(AA) into α,*ω*-dithio poly(AA). The dithio-terminated poly(AA) precursor was recovered by adding 0.1 M HCl into the reaction mixture, then water was removed, the residue was dissolved in THF, and salts were removed by filtration. Finally, the α,*ω*-dithio poly(AA) was precipitated into diethyl ether and collected by filtration.

Preparation of Reducible Vinyl Polymers via Oxidation of α,*ω*-Dithio-Terminated Polymers. The typical oxidation procedure for thiol-functionalized St or AA blocks was as follows. A dithio-functionalized polymer (100 mg) was dissolved in THF (4.0 mL) containing I_2 (50.0 mg). After the mixture was stirred for a certain time at room temperature, the mixture was concentrated by removing THF. The reducible polymer was obtained by precipitation and filtration.

Because PDMAEMA can complex with I_2 and $FeCl_3$, reducible PDMAEMA was prepared by oxidation of dithio-functionalized poly-(DMAEMA) with dimethylsulfoxide (DMSO) or oxygen in water. The detailed procedure was as follows: α,*ω*-dithio poly(DMAEMA) (100 mg) was dissolved in water (9.0 mL), DMSO (1.0 mL) was added after DMAEMA polymer completely dissolved in water, and the reaction mixture was stirred for 13 days. Then, water was removed and reducible PDMAEMA was precipitated from DMSO.

Results and Discussion

The detailed procedure for preparation of the reducible vinyl polymers is outlined in Scheme 1. First, a difuctional RAFT agent was synthesized by the addition reaction of 1,4-diisopro-

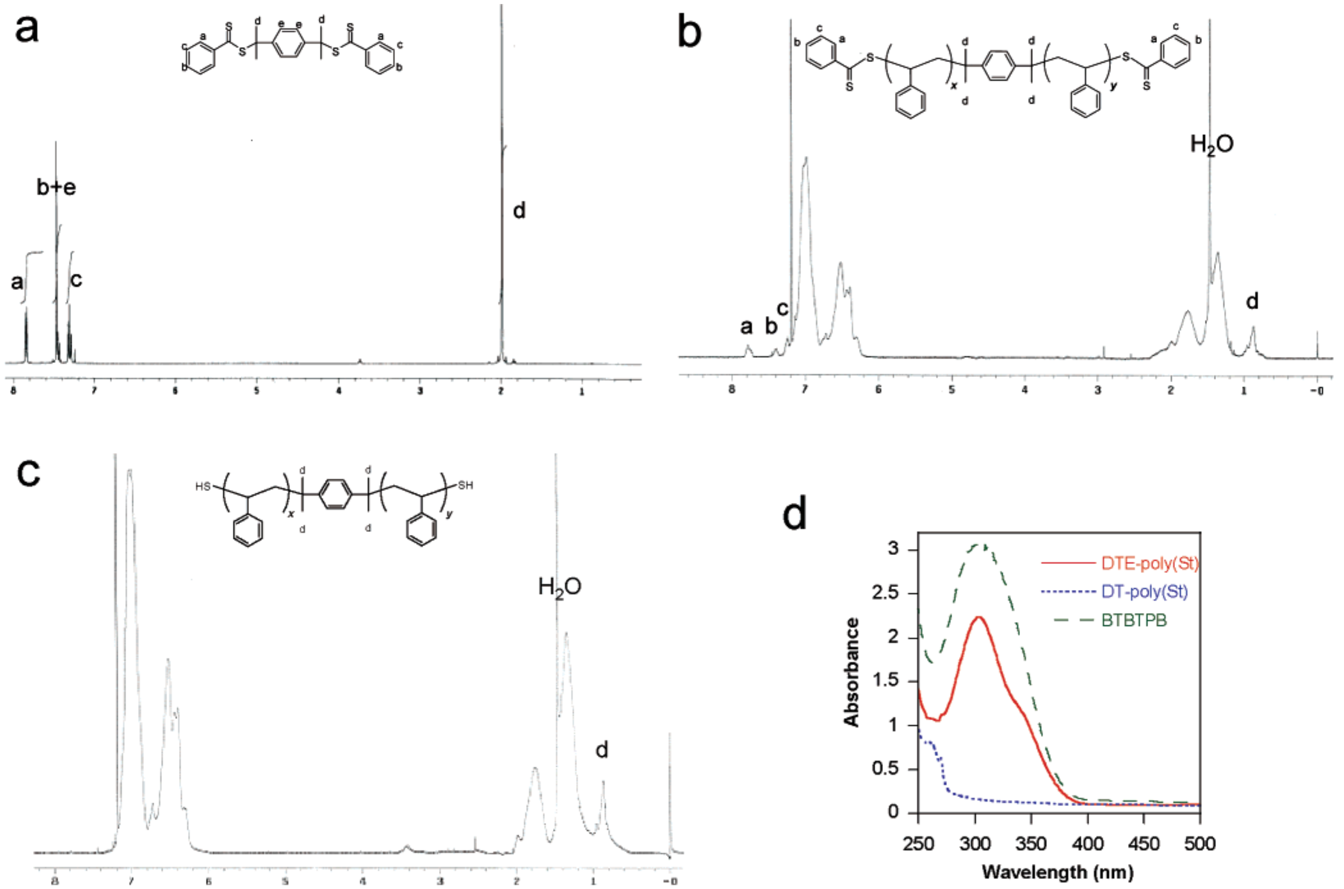


Figure 2. Synthesis of reducible poly(St). ^{1}H NMR spectra of (a) BTBTPB, a difuctional RAFT agent, (b) α,ω-bis(dithioester) poly(St), and (c) α,ω-dithio poly(St). (d) UV absorption of the BTBTPB transfer agent, α,ω-bis(dithioester) poly(St) (DTE-poly(St)) and α,ω-dithio poly(St) (DT-poly(St)).

penylbenzene (**1**) with DTBA.[27] The obtained 1,4-bis(2-(thiobenzylthio) prop-2-yl)benzene (**2**, BTBTPB) RAFT agent was then used in AIBN-initiated radical polymerization to obtain α,ω-bis(dithioester)-functionalized polymers (**3**). Treatment of the α,ω-bis(dithioester) precursors with hexylamine or NaOH yielded homobifunctional α,ω-dithio-functionalized polymers (**4**). Finally, reducible vinyl polymers (**5**) containing multiple disulfide bonds in the backbone were prepared by oxidation of the α,ω-dithio-functionalized blocks using oxidation agents such as I_2 or DMSO.

The approach to the linear reducible vinyl polymers described here has a number of potential advantages, including: (i) its versatility due to the suitability of RAFT polymerization to a wide range of vinyl monomers; (ii) the chain length of the building blocks can be well controlled, and thus the chain length between the neighbor disulfide bonds can be controlled; (iii) the residue from selected RAFT agents remaining in the center of the building blocks can be utilized for further selective functionalization.[32] The versatility of this approach is demonstrated in the example of three different common monomers of both acrylate and methacrylate nature.

RAFT polymerization of St was carried out at 60 °C using BTBTPB as a RAFT agent, producing poly(St) with dithioester groups at both chain ends as shown in Scheme 1. The high functional purity of the bis(dithioester)-terminated precursors is of key importance for the preparation of high-molecular-weight reducible polymers.[29] The synthesized α,ω-bis(dithioester)-functionalized poly(St) was characterized by SEC, NMR, and UV spectroscopy. Polymerization kinetic results show that the molecular weight of the synthesized α,ω-bis(dithioester) poly(St) increased linearly with St conversion (Figure 1). The number-average molecular weight of the polymers obtained from SEC (M_n(SEC)) agreed well with the number-average molecular weight obtained from ^{1}H NMR (M_n(NMR)) as shown in Table 1, confirming the expected difunctionality of the synthesized poly(St). The molecular weights obtained from SEC and NMR also were in close agreement with the theoretical values. Figures 2a and 2b show ^{1}H NMR spectra of BTBTPB difuctional RAFT agent and the synthesized poly(St) precursors. The presence of the terminal dithioester groups is confirmed by the signals at 7.3−8.0 ppm. It is well-known that the reaction between dithioesters and amines proceeds rapidly at ambient temperature and leads irreversibly to thioamides and thiols.[24,27,28,30] As expected, therefore, aminolysis of α,ω-bis(dithioester) poly(St) with hexylamine yielded α,ω-dithio poly(St) blocks. The conversion was first confirmed by the disappearance of the aromatic protons of terminal dithioester groups (Figure 2c). This was further verified by the disappearance of the dithioester group absorption at 305 nm (Figure 2d).

The above synthetic approach was extended to a polymerization of two biomedically important monomers, a cationic DMAEMA and an anionic AA. DMAEMA copolymers are investigated as carriers in nonviral gene delivery.[33−35] Similar to the preparation of α,ω-dithioester-functionalized poly(St), well-defined homobifunctional α,ω-bis(dithioester) poly(DMAEMA) and poly(AA) precursors were prepared by RAFT polymerization using the BTBTPB difunctional transfer agent (Figures 3 and 4). Similar to the poly(St), the presence of

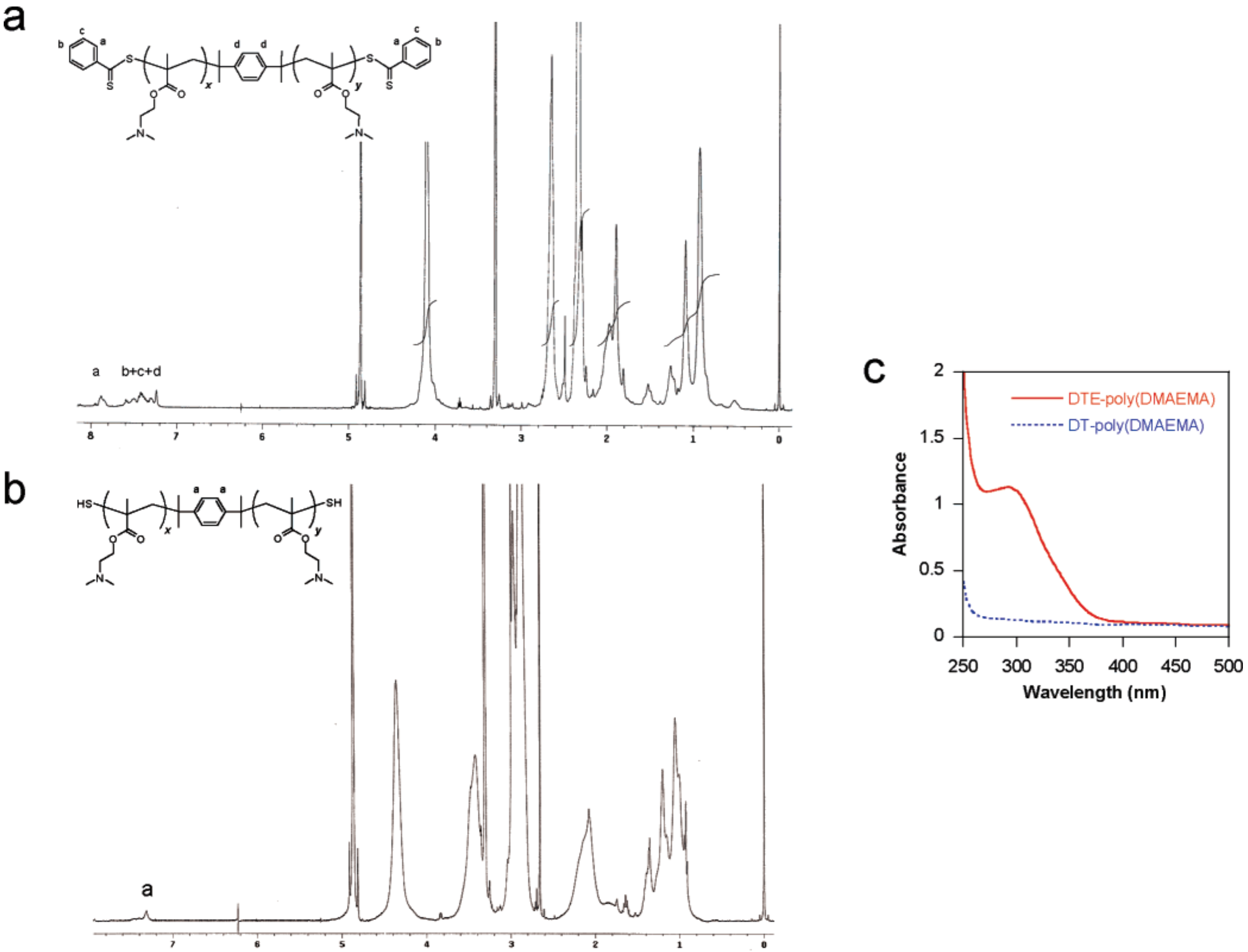


Figure 3. Synthesis of reducible poly(DMAEMA). ^{1}H NMR spectra of (a) α,ω-bis(dithioester) poly(DMAEMA) and (b) α,ω-dithio poly(DMAEMA). (c) UV absorption of α,ω-bis(dithioester) poly(DMAEMA) (DTE-poly(DMAEMA)) and α,ω-dithio poly(DMAEMA) (DT-poly(DMAEMA)).

terminal dithioester functionalities and their conversion to thiol groups were confirmed by a combination of ^{1}H NMR and UV−vis spectroscopy.

Oxidation of terminal thiol groups to form high-molecular-weight reducible polymers with disulfide bonds in the backbone has been widely exploited in the preparation of peptide-based materials.[17,36] In addition to direct oxidation, alternative methods of preparation of such reducible polymers have been reported that rely on the use of disulfide-containing monomers.[37−39] Here, we have successfully used the direct oxidation of terminal thiol groups to prepare reducible polymers based on St, DMAEMA, and AA (Table 1). To prepare reducible poly(St), I_2 was used to polymerize the α,ω-dithio poly(St) blocks. Representative results for I_2 oxidation of the poly(St) blocks are shown in Figure 5. Oxidation of α,ω-dithio poly(St) ($M_n = 3500$) resulted in the formation of reducible poly(St) with $M_n = 35\,200$ (PDI $= 2.3$) after 36 h of reaction. Even after the 36 h reaction time, however, signs of the residual starting precursor were still evident. The synthesized reducible poly(St) was then subjected to reduction with dithiothreitol (DTT) at room temperature. The molecular weight decreased to 4100 as shown in Table 1 and Figure 6, clearly indicating an efficient reduction of the reducible polymer.

Reducible polymers also were successfully synthesized from the α,ω-dithio-terminated poly(DMAEMA) and poly(AA). Several oxidation protocols were explored in the preparation of these two polymers. Reducible poly(DMAEMA) could be efficiently prepared by oxidation with oxygen or DMSO, while other tested agents such as I_2 and $FeCl_3$ failed to produce the desired polymers, most likely due to complexation with the dimethylamino groups. Representative results for oxidation of poly(DMAEMA) are shown in Table 1. Reducible poly(DMAEMA) with a molecular weight of 21 000 (PDI $= 1.8$) was synthesized from α,ω-dithio poly(DMAEMA) blocks with a molecular weight of 2900. Reduction with DTT resulted in a molecular weight decrease to 2600, indicating, as in the case of reducible poly(St), an efficient reduction of the disulfide bonds. Oxidation of the α,ω-dithio poly(AA) precursor ($M_n = 2500$) was conducted using I_2 and afforded anionic reducible poly(AA) with $M_n = 32\,000$ (PDI $= 2.5$). The reducibility was again confirmed by treatment with DTT, yielding a product with a molecular weight close to that of the original poly(AA). A comparison with a recent study shows that the reducible poly(St) synthesized here had a significantly higher molecular weight than that previously reported.[30] Our data suggest that reducible poly(St) consisted of 10 poly(St) blocks, the reducible poly(DMAEMA) consisted of 7, and reducible poly(AA) consisted of 13 of the respective building blocks. While this confirms successful formation of the reducible polymers, the number of the blocks connected together was generally lower than that of the previously reported peptide-based reducible

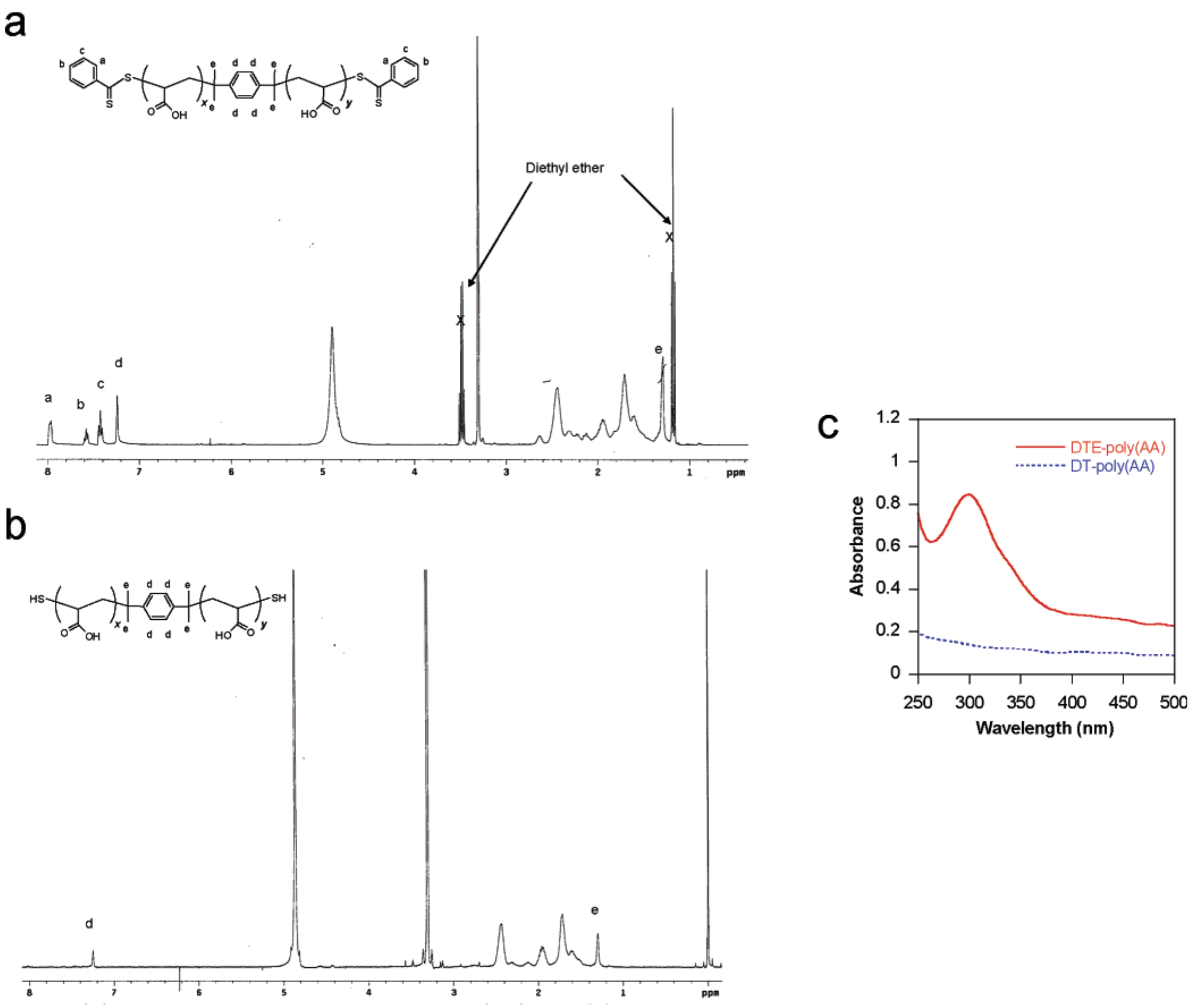


Figure 4. Synthesis of reducible poly(AA). ^{1}H NMR spectra of (a) α,ω-bis(dithioester) poly(AA) and (b) α,ω-dithio poly(AA). (c) UV absorption of α,ω-bis(dithioester) poly(AA) (DTE-poly(AA)) and α,ω-dithio poly(AA) (DT-poly(AA)).

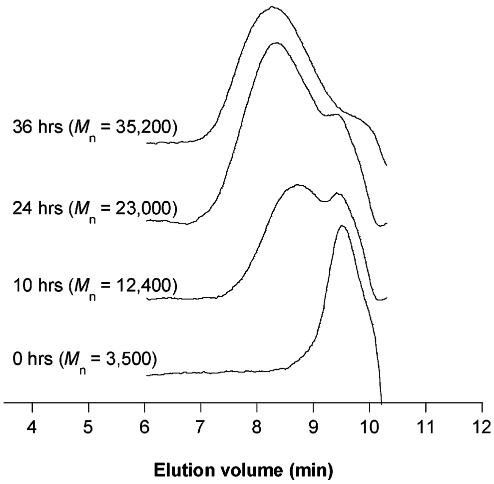


Figure 5. SEC traces of reducible poly(St) during oxidation of α,ω-dithio poly(St) precursors with I_2.

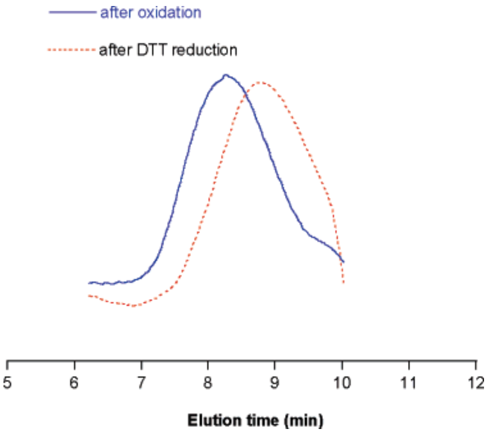


Figure 6. SEC traces of reducible poly(St) (A) before and (B) after reduction with 10 mM DTT.

polymers, which consisted of up to 80 oligopeptide blocks.[40] Additional optimization of the RAFT polymerization conditions is required to further enhance the molar mass of the reducible polymers.

Conclusions

A difunctional RAFT agent was used to prepare α,ω-bis-(dithioester)-functionalized polymers of St, DMAEMA, and AA. The terminal thioester groups were aminolyzed to produce thiol groups, which were subsequently subjected to an oxidation, resulting in the synthesis of linear, reducible polymers. The reducible polymers could be efficiently reduced into starting building blocks. The results reported here suggest that telechelic polymers prepared by RAFT polymerization can be applied to the synthesis of a variety of reducible polymers, including reducible polycations and polyanions. Because of the nature of the oxidation reaction, this approach can be extended to the preparation of functional multiblock copolymer materials consisting of different polymeric building blocks.[18,41] Overall, the described materials have the potential to substantially increase the portfolio of available reducible polymers for use in a wide variety of biomedical applications. In particular, drug and gene delivery will benefit from the anticipated reduced toxicity and improved body elimination of shorter (meth)acrylate polymeric fragments generated after reduction.[42−44]

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